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Copper deficiency halves serum dehydroepiandrosterone in rats

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Summary

DHEA (dehydroepiandrosterone) is a hormone often taken as a dietary supplement to prevent the normal decline with age and in the hope of preventing heart attacks. Rats in two experiments were made deficient in copper by standard methods and criteria. Copper deficiency decreased DHEA in serum by approximately 50%. People who associate higher serum concentrations of DHEA with health probably should eat a diet adequate in copper.

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Introduction

Dehydroepiandrosterone (DHEA), and its sulfated form, DHEAS, are hormones synthesized by the adrenal cortex and the gonads. Peak synthesis occurs in humans early in adult life with plasma concentrations reflecting adrenal output. There is a general decline in secretion with age; in adults very low concentrations are found after age 70. DHEA may protect people from atherosclerosis or acute myocardial infarction; effects seem greater in men than in women.

Numerous biological effects of DHEA have been discovered including increased heat production, inhibition of platelet aggregation and stimulation of immune function. DHEA can stimulate the activity of several enzymes including catalase, glycerol-3-phosphate dehydrogenase and malic enzyme. Its action may be mediated by androgens, estrogens and thyroid hormone.

Interest in DHEA is increasing among medical scientists (1-4) and lay people, alike (5-7). Administration of DHEA can alter metabolism in many ways including effects on enzymes, food intake, hormones, lipids, proteins, and weight (2-4). That nutritional status may alter DHEA metabolism generally seems not to have been considered, and has been noticed only rarely. For example, DHEA decreased LDL cholesterol only when monkeys were fed a low-fat diet (4) (their reference 74).

Copper is an essential nutrient with effects on many metabolic processes (8, 9). Free copper ion does not exist in biological systems where copper is bound to proteins and other ligands in a variety of oxidative states. As synthesis of DHEA and other steroid hormones from cholesterol requires oxidative transformation (10), and as all the enzymes that require copper for activity are oxidative (11), it was decided to test the hypothesis that copper deficiency can decrease the concentration of DHEA in rat serum.

Materials and Methods

Animals[3] and diets

Twenty male, weanling Sprague-Dawley rats (Sasco, Omaha, NE)[4] were used in each of two experiments after being matched into equal groups by mean weights: 49.9g (experiment 1) and 60.8g (experiment 2). Animals received purified diet

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[3]Use of animals was approved by the Animal Care and Use Committee of the Center according to the National Institutes of Health Guide for Care and Use of Laboratory Animals.
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based on sucrose (62%), egg white protein (20%) and corn oil (10%) *ad libitum* for 5 weeks (12) and were weighed weekly. Environmental conditions were similar to those described (13). The diet contains all nutrients known to be essential for rats and has been in use for more than two decades (14), but is deficient in copper and zinc without supplementation. Supplemented rats received a solution containing 10 mg Zn/ml (as acetate) and 3 mg Cu/ml (as sulfate); deficient rats received only 10 mg Zn/ml.

Blood and organ analyses

Plasma cholesterol was determined by the method of Carpenter, et al. (15) from blood collected from the tail vein into heparinized tubes. When hypercholesterolemia was detected after 35 days, rats were anesthetized with intra peritoneal ketamine hydrochloride and xyzaline. Blood was collected for DHEA analysis from the vena cava, chilled on ice and allowed to clot. Serum was stored at -20°C until analysis. Some liver was dissected and frozen. DHEA was measured by radioimmunoassay according to instructions of the manufacturer (DPC, Los Angeles, CA) except that samples were extracted three times instead of once (16). Copper was measured in organs and diet by atomic absorption spectrometry after organic matter was destroyed by nitric acid, sulfuric acid and hydrogen peroxide (17). Data were analyzed by t-test or the Wilcoxon two-sample test (9).

Results

Two deficient rats in each experiment died prematurely with gross pathology. All four had atrial thrombosis. In experiment one, one rat had ascites; the other had hydrothorax plus ventricular aneurysm. In experiment two, both rats had pleural effusion; one also had ascites.

Copper deficiency decreased hematocrit and increased cholesterol in plasma in both experiments (Tables 1 and 2). Liver copper was decreased in deficiency.

Blank samples gave DHEA values below the detection limits of the assay. Replicate analyses of a standard revealed recovery exceeded 99% with a coefficient of variation of 4%. Values on supplemented rats were similar to those published by others.

Because serum DHEA values were positively skewed in experiment one, they were compared by the Wilcoxon two-sample test. Median, deficient values were approximately half supplemented ones, 0.60 and 1.17 μg/L, respectively ($p<0.04$, $n=18$). Removal of one value in each group which was more than two SD from the mean resulted in similar values (Table 1). Table 2 contains data from experiment two, which were confirmatory, because skewing was not prominent, only the t-test was used.

Table 1. Biochemistry and Hematology, Experiment 1

	Cu		
	Deficient	Supplemented	P
Cholesterol (mg/dl)	135±7.4	114±6.4	<0.05
Hematocrit (%)	38±3.4	53±1.3	<0.0025
Hepatic copper (mg/g, dry)	1.7±0.20	8.2±0.33	0.0001
Serum DHEA (μg/L)	0.55±0.08	1.18±0.19	<0.02

$\bar{x}$ ± SE, 10 supplemented and 8 deficient rats, except DHEA values of 9 and 7 respectively.

Discussion

Copper deficiency was verified indirectly by anemia and hypercholesterolemia (11, 18) and pathology suggestive of copper deficiency. Atrial thromboses (19), pleural effusion (19, 20) and ventricular aneurysms (20, 21) are common in copper deficiency. When animals fed a diet low in copper exhibit these phenomena, these signs are diagnostic of copper deficiency. That is, a bioassay of the diet has been done. A decrease in liver copper of approximately 80% provided a direct verification of deficiency.

The concentration of DHEA in serum was decreased approximately 50% by copper deficiency. The hypothesis was tested successfully. The second experiment was done to confirm the results of the first because of our realization that the results may surprise some biologists.

Our experimental plan included the collection of some organs with the hope of measuring DHEA in them and identifying anatomical location(s) of metabolic change(s) to explain anticipated differences in serum concentrations. Unfortunately, none of the samples survived the great flood of 1997 which severely damaged our Center and during which all of the population of Grand Forks was evacuated for several weeks. Thus, there was no opportunity to evaluate steroid interconversions. Liver copper in experiment one had been measured earlier.

Svec and Porter (4) summarized apparently contradictory effects on plasma lipids of DHEA given to humans and hinted that diet (e.g., fat) may have affected results. Perhaps the subjects of the studies had heterogeneous status in regard to copper nutriture.

Diets in the U.S. and other industrialized nations frequently are low in copper in comparison to reference intakes and

Table 2. Biochemistry and Hematology, Experiment 2

	Cu		
	Deficient	Supplemented	P
Cholesterol, mg/dl	99±11.1	71±3.7	<0.05
Hematocrit, %	45±4.2	58±1.3	<0.02
Serum DHEA, ng/ml	0.60±0.12	1.07±0.17	<0.05

$\bar{x}$ ± SE, 10 supplemented and 8 deficient rats.

amounts that have proved insufficient for men and women in controlled experiments (22). People with lower concentrations of DHEA are more likely to be low in copper than those with higher concentrations if people respond to diets low in copper similarly to rats. Those who believe that high concentrations of DHEA are beneficial may wish to increase their intake of copper by substituting foods higher in copper for foods lower in copper (23). Supplementation with copper is likely to be less hazardous and less expensive than supplementation with DHEA.

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